REMARKS

With entry of this amendment, Claims 1-6 are pending. No claim amendments have been made. The amendments to the specification reflect the request of the Examiner to properly indicate trademarked terms and include the generic definitions of these terms. No new matter has been added by these amendments.

35 U.S.C. §103(a)

Claims 1 and 4 are rejected under 35 U.S.C. §103(a) as being unpatentable over Johansson *et al.* in view of MacDonald *et al.* The Office Action states "Johansson *et al.* teach a method of purifying recombinantly produced periplasmic protein, exotoxin A, . . .comprising growth of the target protein, . . . applying a mixture containing the protein to an expanded bed cation exchange Streamline™ column, . . .collecting eluate from the expanded bed cation exchange column, purifying the cells, concentrating, filtering, passing through a membrane . . and applying the eluate to a Sepharose column . . . and later to a hydrophobic column." MacDonald *et al.* "teaches the advantages of recombinant production of angiostatin protein." The Office Action goes on to state that "given the advantages of large scale purification of angiostatin and the purification otherwise done by centrifugation, namely, purification of angiostatin, could also be purified by the more advantageous expanded bed methods, it would have been obvious to one of ordinary skill in the art at the time of the invention to use expanded bed chromatography to produce high yield angiostatin protein." Applicants traverse this rejection.

Johansson *et al.* teaches a method of purifying recombinantly produced periplasmic protein, exotoxin A, comprising treating thawed bacterial cells suspended in sucrose and Tris buffer with Dnase and then pumping the suspended cells upward into an expanded bed cation exchange Streamline™ bed column. The eluent from the Streamline™ column is then applied to a Phenyl Sepharose 6 Fast Flow (hydrophobic) column. The eluent from the phenyl sepharose pool is then applied to a SOURCE 30Q™ (anion exchange) column and the fractions containing exotoxin A activity are pooled. In contrast, Applicants Claim 1 comprises applying crude

fermentation broth containing the recombinantly produced angiostatin to an expanded bed cation exchange column; collecting eluate from the expanded bed cation exchange column and applying the eluate from the expanded bed cation exchange column to an anion exchange column; collecting eluate from the anion exchange column and applying the eluate from the anion exchange column to a hydroxyapatite column; collecting eluate from the hydroxyapatite column and applying the eluate from the hydroxyapatite column to a hydrophobic column; collecting eluate from the hydrophobic column and applying the eluate from the hydrophobic column to a membrane; and collecting fluid passing through the membrane. Johansson *et al.* does not suggest, teach or disclose the use of a hydroxyapatite column as claimed. Furthermore, Johansson *et al.,* applies the suspension to a hydrophobic column prior to applying it to an anion exchange column. Johansson *et al.,* therefore alters the order of the steps in the purification process as well as omitting a filtration step required by the present invention as claimed. There is no teaching or suggestion in Johansson *et al.* that further purification such as with a hydroxyapatite column or an alteration in the order of filtration would be necessary or desirable.

MacDonald *et al.* teaches the utility of producing large quantities of recombinant human Angiostatin protein, however the only purification method disclosed is centrifugation. There is no teaching or suggestion that other methods of purification would be effective.

While Johansson *et al.* states that expanded bed chromatography is a possible alternative to centrifugation, it does not state that expanded bed chromatography is equivalent to centrifugation. It would therefore require undue experimentation for one of ordinary skill in the art to determine whether recombinant angiostatin protein could be purified by the method used in Johansson *et al.* for exotoxin A. Even if there were a motivation to combine Johansson *et al.* and MacDonald *et al.,* the resulting method of purification would not include the hydroxyapatite column required by the present invention as claimed and would provide a different order of steps. Accordingly, the present invention is not disclosed, taught or suggested by Johansson *et al.* in view of MacDonald *et al.* Applicants respectfully assert that the rejection under 35 U.S.C. §103(a) has been overcome and request its withdrawal.

35 U.S.C. §103(a)

Claims 2 and 3 are rejected under 35 U.S.C. §103(a) as being unpatentable over Johansson *et al.* in view of MacDonald *et al.,* and further in view of Goldstein *et al.* The Office Action states "Goldstein *et al.* teach the purification of proteins by concentrating the proteins, . . . diafiltration . . .and then further concentration of the proteins, . . . purification by ammonium sulfate, . . . by hydrophobic chromatography, . . . hydroxylapatite chromatography, . . . by passing through more than one membrane, . . . concentrating and pooling the fluid, and aseptically filtering the concentrated fluid." The Office Action goes on to state that Goldstein *et al.* teaches that these steps prevent the contamination of the proteins during the purification process and it would have been obvious to one of ordinary skill in the art to use these steps to purify recombinantly produced angiostatin. Applicants traverse this rejection.

As argued above, Applicants respectfully assert that there is no motivation to combine Johansson *et al.* with MacDonald *et al.*, and even if they were combined, they would not result in the present invention as currently claimed. There is no teaching or suggestion to alter the steps of the purification process nor is there any teaching or suggestion in Johansson *et al.* that the method they disclose is incomplete. Therefore, there is no motivation provided by these references to add any additional purification steps such as the hydroxyapatite column or to alter the steps so that the solution is applied to an anion exchange column before application to a hydrophobic column as claimed.

Goldstein *et al.* does not teach or suggest the use of expanded bed chromatography. Therefore, there is no motivation to combine Goldstein *et al.* with the method of Johansson *et al.* In Goldstein *et al.* the bacterial cells which serve as the source for the thermostable enzymes are treated to strip away the cell walls and covert the cells to spheroplasts (Column 6, lines 53-56). Following permeabilization of the bacterial cells, the spheroplasts are subjected to microfiltration to release the enzymes and remove particulate matter, concentration of the microfiltrate and diafiltration. (Column 8, lines 21-25). Following concentration or diafiltration, the enzymes may

be purified by a variety of purification techniques (Column 9, lines 21-25). Goldstein *et al.* discloses several different purification techniques including hydrophobic interaction chromatography and hydroxyapatite chromatography. In Example 3 of Goldstein *et al.*, cited by the Office Action, the filtrate is passed over a Fractogel column. The Fractions were pooled and then purified with a single-stranded DNA agarose column, followed by a ceramic hydroyapatite column, AF-Heparin-650M affinity column, and dialysis. (See Example 3, columns 12-13 of Goldstein *et al.*).

Even if Goldstein *et al.* were combined with MacDonald *et al.* and Johansson *et al.*, it would not result in the present invention as currently claimed. The combination of MacDonald *et al.* with Johansson *et al.* and Goldstein *et al.* would result in a method of filtration in which expanded bed filtration was followed by a hydrophobic column and a SOURCE 30Q™ column. The resulting eluate would then pass through a single-stranded DNA agarose column, a ceramic hydroxyapatite column and an AF-Heparin-650M column. There is no teaching or suggestion in MacDonald *et al.*, Johansson *et al.*, or Goldstein *et al.*, alone or in combination, to select the particular sequence of steps used in the methods claimed in the current invention. The mere fact that the different types of purification exist would not lead one of ordinary skill in the art to combine them as recited in the claims of the present invention. Applicants respectfully assert that the rejection under 35 U.S.C. §103(a) has been overcome and request its withdrawal.

35 U.S.C. §103(a)

Claim 5 is rejected under 35 U.S.C. §103(a) as being unpatentable over Johansson *et al.* in view of MacDonald *et al.* and further in view of Folkman *et al.* The Office Action states that Folkman *et al.* teach "a method of expressing human angiostatin in *Pichia pastoris* ... and that the advantages thereof in terms of protein processing, protein folding, and posttranslational modification inclusive of glycosylation, ... and also teach a method of purifying recombinantly produced angiostaton comprising applying crude fermentation broth containing the recombinantly produced angiostain and isolated over sepharose .... Given the advantages of expressing human angiostatin in *P. pastoris* as taught by Folkman *et al.*, it would have been obvious to one of

ordinary skill in the art at the time of the invention by the applicant to purify recombinantly produced angiostatin that is produced from fermentation of *P. pastoris*." Applicants traverse this rejection.

As argued above, Applicants respectfully assert that there is no motivation to combine Johansson *et al.* with MacDonald *et al.*, and even if they were combined, they would not result in the present invention as claimed. The expanded bed chromatography method disclosed by Johansson *et al.* does not include the hydroxyapatite column as claimed. Furthermore, the ion exchange step performed in Johansson *et al.* occurs after the hydrophobic chromatography step, not before as recited in the claims of the present invention. Johansson *et al.* therefore performs purification in a different order and omits one of the steps recited in the claims. MacDonald *et al.* teaches the advantages of recombinant production of angiostatin protein. The Office Action states "Folkman *et al.* teach a method of expressing human angiostatin in *Pichia pastoris*." However, neither MacDonald *et al.* nor Folkman *et al.* cure the missing step or reordered purification in the method disclosed in Johansson *et al.* Furthermore, there is no motivation, teaching, or suggestion to combine Johansson *et al.*, directed to purification of exotoxin A from *E. coli*, with MacDonald *et al.*, which discloses the tumor-suppressing activity of angiostatin protein, or Folkman *et al.*, which discloses a method of expressing human angiostatin. Even if they were combined, they would not teach, disclose, or suggest the method of the present invention as claimed. Applicants respectfully assert that the rejection under 35 U.S.C. §103(a) has been overcome and request its withdrawal.

35 U.S.C. §103(a)

Claim 6 is rejected under 35 U.S.C. §103(a) as being unpatentable over Johansson *et al.* in view of MacDonald *et al.*, and further in view of Flickinger *et al.* The Office Action states that given the benefits of following standard and known procedures for expanded bed chromatography, for a successful outcome as taught by Flickinger *et al.* would have been obvious to one of ordinary skill in the art at the time of the invention to follow standard expanded bed chromatography procedures and to apply fluid with the angiostatin to the expanded bed in an

upward direction and the elution buffer in the reverse direction. Applicants traverse this rejection.

As argued above, Applicants respectfully assert that there is no motivation to combine Johansson *et al.* with MacDonald *et al.*, and even if they were combined, they would not result in the present invention as claimed. The expanded bed chromatography method disclosed by Johansson *et al.* does not include the hydroxyapatite column claimed. Furthermore, the ion exchange step performed in Johansson *et al.* occurs after the hydrophobic chromatography step, not before as recited in the claims. Johansson *et al.* therefore performs purification in a different order and omits one of the steps recited in the claimed invention. MacDonald *et al.* teaches the advantages of recombinant production of angiostatin protein. The Office Action states that "Flickinger *et al.* teach that in expanded bed chromatography used to purify proteins, it is standard that the fluid containing the protein to be purified is passed through the column in one direction and the elution buffer flow in the reverse direction." However, Flickinger *et al.* does not teach, disclose or suggest that further purification steps, as claimed in the present invention, are necessary or desirable after expanded bed chromatography. Furthermore, there is no motivation to combine Johansson *et al.* with MacDonald *et al.* and Flickinger *et al.* Even if they were combined, they would not teach the present invention as claimed. Applicants respectfully assert that the rejection under 35 U.S.C. §103(a) has been overcome and request its withdrawal.

Applicants respectfully submit that this is a complete response to the Office Action dated March 14, 2003 and that Claims 1-6 are patentable. Early and favorable consideration is earnestly solicited. If the Examiner believes there are other issues that can be resolved by telephone interview, or that there are any informalities remaining in the application, which may be corrected by Examiner's Amendment, a telephone call to the undersigned attorney at (404) 815-6500 is respectfully solicited.

Respectfully submitted,

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